



National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material[®] 927d

Bovine Serum Albumin (7 % Solution)

(Total Protein Standard)

This Standard Reference Material (SRM) is intended primarily for use in the standardization of procedures employed in clinical analyses for total serum protein, for critical evaluation of daily working standards used in these procedures, and as a reference standard for assays of total protein by colorimetric methods. This SRM is a solution (mass fraction 7 %) of known protein concentration and purity. It conforms to the specification for standardized protein solution approved by the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) [1]. The protein content of this SRM was determined using the biuret reference method [2] that is recommended for use in standardizing laboratory-prepared protein solutions and “normal” serum pools. Such standardized “normal” sera could then be used to calibrate refractometers or other instruments for serum protein estimations. SRM 927d may also be used for other procedures, such as gel diffusion, amino acid analysis, electrophoresis, nitrogen assays, or other tests that require well-characterized protein for calibration or evaluation. A unit of 927d consists of 10 ampoules, each containing 2.2 mL of solution.

In addition to the measurements using the biuret method, NIST made measurements of the bovine serum albumin (BSA) concentration using amino acid analysis. There is a discrepancy between the results from the two approaches. As the two different approaches are not determining the same measurand, it was decided to separately report the results from the two approaches as follows: 1) certified BSA concentration by amino acid analysis and 2) reference total protein concentration by the biuret method.

Certified Bovine Serum Albumin (BSA) Concentration and Uncertainty from Amino Acid Analysis: Two different methods of amino acid analysis were used to measure the concentration of bovine serum albumin (see “Analytical Methods” section). The certified results are shown in Table 1. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or accounted for by NIST.

Reference Total Protein Concentration and Uncertainty as Determined Using the Biuret Method: The biuret reference method [2] was employed to determine protein concentration in SRM 927d using SRM 927c as an external standard. The reference protein concentration and uncertainty are shown in Table 2. Reference values are noncertified values that are the best estimates of the true values; however, the values do not meet NIST criteria for certification and are provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods.

Additional Reference Values and Uncertainties: Reference values are provided in Table 3 for additional properties including fill volume, pH, density, absorbances at various wavelengths, and relative molecular mass as determined using electrospray ionization mass spectrometry.

Expiration of Certification: This certification is valid until **30 September 2010**, within the measurement uncertainties specified, provided the SRM is handled and stored in accordance with the instructions given in this certificate. However, the certification will be nullified if the SRM is contaminated or modified.

Stephen A. Wise, Chief
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Gaithersburg, MD 20899
Certificate Issue Date: 02 February 2006

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Information Value: A literature value for the optical absorbance of bovine serum albumin is given in Table 4. This is a noncertified value with no reported uncertainty as there is insufficient information to assess uncertainty. This information value is given to provide additional characterization of the material.

The overall direction and coordination of technical measurements leading to the certification were performed by M.J. Welch and D.M. Bunk of the NIST Analytical Chemistry Division.

Analyses were performed by D.M. Bunk, L.T. Sniegowski, and M. Vergne of the NIST Analytical Chemistry Division.

The statistical analysis of the data used for certification was performed by N.F. Zhang of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Measurement Services Division.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its value assignment. If substantive technical changes occur that affect the value assignment before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

NOTICE AND WARNING TO USERS

SRM 927d IS INTENDED FOR IN-VITRO DIAGNOSTIC USE ONLY. The blood used in the preparation of SRM 927d Bovine Serum Albumin (7 % Solution) was collected from cattle sourced in the United States. Only blood from cattle/carcasses that have passed ante-mortem and post-mortem USDA-Food Safety Inspection Service (FSIS) inspection was used. This material was collected prior to any known case of Bovine Spongiform Encephalopathy (BSE) in the United States. There were no additives to the pooled serum prior to protein purification.

INSTRUCTIONS FOR USE

Storage: This SRM is supplied to the user in sealed ampoules. The SRM should be stored in a refrigerator at a temperature between 2 °C and 8 °C. The ampoules should not be frozen because of possible breakage of ampoules during the thawing process. Once an ampoule is opened, the solution should be used promptly. Any unused solution in opened ampoules should be discarded.

Inappropriate Uses: This SRM is not intended to be used as a standard for dye-binding tests, for checking precalibrated refractometers, for immunochemical methods, or as an additive for bilirubin standardization.

SOURCE, PREPARATION, AND ANALYSIS¹

Source of Material: The bovine serum albumin solution was prepared by Bionostics Inc., (Acton, MA, USA) under contract with Bioreclamation, Inc., (Hicksville, NY, USA). The bovine serum was produced for manufacture into products for pharmaceutical use by West Laboratories, Inc. at USDA EST. #245-J, Iowa Beef Packers, Inc., (Joslin, IL, USA).

The BSA for this SRM was dissolved in 0.02 mol/L sodium chloride and the pH adjusted to 6.5 – 6.8 with 1.0 mol/L sodium hydroxide. The material was sterilized by membrane filtration and tested for sterility by approved methods [3].

Preparation of Dilutions: Protein solutions of lower concentration may be prepared by transferring the appropriate aliquot to a volumetric flask and diluting to volume. Diluents are not furnished with the SRM; however, an aqueous sodium chloride diluent, such as a solution having a concentration of 0.15 mol/L, may be used.

¹Certain commercial equipment, instruments, or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Analytical Methods: All analyses for the certified and reference values were performed at NIST. For the determination of BSA, two different methods for determining amino acids were used. The first method involved isotope dilution liquid chromatography/tandem mass spectrometry (ID-LC/MS/MS). Samples of SRM 927d and 927c (as a control) were hydrolyzed with vapor phase hydrochloric acid (HCl) for 2 h at 150° C in sealed vials. After hydrolysis, the samples were lyophilized and then reconstituted with 0.2 % formic acid in acetonitrile containing isotope labeled analogs of phenylalanine, proline, and valine. Amino acids were separated using hydrophilic interaction chromatography (HILIC) on a polyhydroxyethyl aspartate column with gradient elution. Measurements were performed on a triple quadrupole mass spectrometer, monitoring specific transitions for each amino acid. The measurements were calibrated with different dilutions of SRM 2389 *Amino Acids in 0.1 mol/L HCl*. Data were collected for phenylalanine, proline, and valine. Based upon the known amino acid sequence for BSA, the concentration of BSA was calculated from the concentrations determined for each of the amino acids. For the second method, a commercial amino acid analyzer was used following hydrolysis as described above. This analyzer uses an ion exchange resin column to separate the amino acids, followed by derivatization with ninhydrin, and detection by spectrophotometry at 570 nm and 440 nm. These measurements were calibrated with dilutions of SRM 2389. The amino acids used for quantitation were alanine, leucine, lysine, and aspartic acid/asparagine.

The reference total protein concentration was measured using the biuret reference method for total serum protein [2]. The measurements involve a direct comparison between the current SRM lot (927d) and the previous lot (927c) and were performed on a Varian Cary 219 spectrophotometer.

The reference values for various properties determined for SRM 927d are given in Table 3. Absorbances were measured in accordance with requirements specified for a standard BSA solution [1]. Measurement of the pH was performed using an Orion Model 501 pH meter with a glass body combination pH electrode calibrated with pH 4 and pH 7 buffers. Density was measured using the Lang-Levey pipet method [4]. Fill masses were determined gravimetrically and fill volumes were calculated from the fill masses and mean density.

Relative molecular mass was determined using liquid chromatography/mass spectrometry (LC/MS). Measurements were performed on a single quadrupole mass spectrometer operated in the positive ion mode and coupled to a capillary LC with a commercial C-4 column, 0.5 mm × 50 mm, held at 40 °C. Gradient elution using 0.05 % trifluoroacetic acid in water and in acetonitrile was used. Horse apomyoglobin was used for mass calibration of the mass spectrometer. The molecular masses of the seven major forms of BSA found in SRM 927d are shown in Table 3 in decreasing order of abundance. The previous lot (SRM 927c) had a similar range of molecular masses.

Table 1. Certified Bovine Serum Albumin Concentration by Amino Acid Analysis^(a)BSA Concentration: 65.41 g/L \pm 0.82 g/L

^(a) The certified value is the equally weighted mean of results obtained from two chemically independent methods (see “Analytical Methods”). The expanded uncertainty in the certified concentration is calculated as $U = ku_c$. The quantity u_c is the combined standard uncertainty calculated based on a Bayesian approach in [5] and ISO and NIST Guides [6]. The coverage factor, $k = 2$, represents an approximate 95 % level of confidence.

Table 2. Reference Total Protein Concentration by the Biuret Method^(a)Protein Concentration: 70.10 g/L \pm 0.74 g/L

^(a) Because only a single method was used, the results do not meet the NIST criteria for a certified value [7]. Therefore this method specific result is considered a reference value. The expanded uncertainty in the reference concentration is calculated as $U = ku_c$. The uncertainty u_c is based upon quadratically combining the measurement uncertainty with the uncertainty in SRM 927c, which served as the external standard for the measurements. The major component in the uncertainty for SRM 927d is the uncertainty in the concentration of SRM 927c. The coverage factor, $k = 2$, represents an approximate 95 % confidence interval.

Table 3. Reference Values for Various Properties of SRM 927d

Mean Fill Volume:	2.245 mL	\pm	0.006 mL
pH:	6.70	\pm	0.01
Density:	1.0178 g/mL	\pm	0.0003 g/mL
Spectral Properties		Absorbance	
Ultraviolet (A_{252}/A_{279} ratio @ 1.0 g/L)	0.467	\pm	0.002
Soret Band (Visible) A_{405}	0.140	\pm	0.002
A_{500}	0.0320	\pm	0.0021
A_{600}	0.0187	\pm	0.0024
Major molecular forms of BSA in decreasing order of abundance			
Relative Molecular Mass			
	66 432	\pm	7
	66 549	\pm	12
	66 473	\pm	14
	66 593	\pm	11
	66 344	\pm	13
	66 222	\pm	14
	66 389	\pm	14

Theoretical BSA relative molecular mass from Amino Acid Sequence [8]: 66 399

The uncertainties in the reference values are calculated as $U = ku_c$. The quantity u_c is the combined standard uncertainty calculated according to the ISO and NIST Guides [6], where u_c is intended to represent the measurement error at the level of one standard deviation. The coverage factor, $k = 2$, represents an approximate 95 % confidence interval.

Table 4. Information Value for Optical Absorbance

Optical Absorbance @ 279 nm for 1 g/L [9] 0.667

REFERENCES

- [1] NCCLS Approved Standard ACS-1; *Specification for Standardized Protein Solution (Bovine Serum Albumin)*, 2nd ed.; National Committee for Clinical Laboratory Standards: Villanova, PA (1979).
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- [7] May, W.; Parris, R.; Beck, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*; NIST Special Publication 260-135; U.S. Government Printing Office: Washington, DC (2000); available at http://www.cstl.nist.gov/nist839/NIST_special_publications.htm.
- [8] Swiss-Prot database, Swiss Institute for Bioinformatics (<http://us.expasy.org/sprot/sprot-top.html>).
- [9] *All About Albumin: Biochemistry, Genetics, and Medical Applications*; Peters, T., Jr.; Academic Press Inc.: San Diego, CA, p. 25 (1995).

Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-6776; fax (301) 926-4751; e-mail srminfo@nist.gov; or via the Internet at <http://www.nist.gov/srm>